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Vaccine 23 (2005) 884–889
Vaccine
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Lack of toxicity of therapy-induced T cell responses against the universal tumour antigen survivin

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Received 10 February 2004; received in revised form 2 August 2004; accepted 4 August 2004
Available online 11 September 2004

Abstract

Prognosis of disseminated melanoma remains gloomy as neither chemotherapeutic nor unspecific immune modulatory approaches were able to improve the overall survival of these patients. Hence, specific immunotherapy has received increasing attention. Disappointing clinical results, however, indicate that the choice of suitable antigens is of special importance. To this end, the inhibitor of apoptosis (IAP) protein survivin, which is over-expressed in several tumours but is largely undetectable in adult tissues, appears to be a promising target for vaccination purposes, since down-regulation or loss of expression is associated with impaired tumour progression. Consequently, five heavily pretreated stage IV melanoma patients were vaccinated with the HLA-A2 restricted survivin$_{96-104}$ epitope presented by autologous dendritic cells (DCs) in a compassionate use setting. Four of these patients mounted strong T cell responses to this epitope as measured by ELISPOT assay. Furthermore, in situ peptide/HLA-A2 multimer staining confirmed that these survivin reactive cells infiltrated both visceral and soft tissue metastases.

Keywords: Melanoma; Survivin; T cell responses

1. Introduction

Current treatment options for stage IV melanoma are mainly experimental since to date none of the established therapies actually improved overall survival [1]. New therapeutic strategies include anti-angiogenesis [2], inhibition of anti-apoptotic [3] or aberrantly activated signal transduction molecules [4] as well as specific immune therapy. Indeed, the induction of tumour-specific cytotoxic T cells by vaccination with specific peptide-pulsed antigen presenting cells resulted in regression of metastatic melanoma lesions [5] in a few patients. Thus, tumour-specific vaccinations hold the promise of successfully harnessing the immune system to fight cancer.

Dendritic cells (DCs) are the most potent antigen presenting cells known [6]. DCs are derived from bone-marrow and reside in non-lymphoid tissues in immature form. After antigen capture and processing, immature DCs migrate via blood or afferent lymph vessels to T cell areas of secondary lymphoid tissues, e.g. the paracortex of lymph nodes. At this stage, DCs have lost the ability to capture and process antigen but have acquired a high capacity to stimulate T cells. The clinical use of DCs for vaccination purposes, however, is still in its beginnings and several critical variables remain to be optimized, including source of precursors, mode of maturation, dose and route of administration, and choice of antigen. Survivin, a member of the inhibitor of apoptosis (IAP) gene

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0264-410X/$ – see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2004.08.007

family has been suggested as a promising target for therapeutic tumour vaccinations. Survivin was initially described as an inhibitor of caspase-3 and -7, but is also involved in cell cycle control since it binds to the centrosome and microtubules during mitosis [7–9]. It is expressed in embryonic and fetal organs, but was reported largely undetectable in most terminally differentiated adult tissues. In fact, survivin is expressed only on thymus cells, $CD34^+$ bone marrow-derived stem cells, basal colonic epithelial cells, as well as endothelial cells following vascular injury [7]. In contrast, a dramatic over-expression of survivin has been demonstrated in a large variety of tumours. These include among others colon, breast, and lung carcinoma, leukaemia, as well as melanoma. Notably, this over-expression is associated with an unfavourable course of disease, including accelerated progression, higher rates of recurrences, increased resistance to therapy, and shortened survival [9–11]. The almost universal expression of survivin among tumours, combined with the fact that its down-regulation or loss of expression as means of immune escape would hamper the progression of the tumour make survivin an ideal target for vaccination [9]. Recently, we and others were able to identify an HLA-A2 restricted epitope of survivin, which is presented in the context of this MHC molecule on the surface of tumour cells of different tissue origin [12,13]. In this regard, it is important to note that a significant proportion of tumour patients harbour spontaneous T cell responses against survivin-derived epitopes [14].

1.1. Patients, materials and methods

1.1.1. Patient eligibility criteria and treatment regimen

All clinical procedures were in accordance with the Declaration of Helsinki and all patients provided informed consent prior to therapy. Stage IV cutaneous or uveal melanoma patients were eligible when their disease was progressive despite at least two different chemo-, immuno- or chemoimmunotherapies. In addition, patients had to be 18 years or older, express HLA-A*0201, and suffer from measurable disease validated by cranial, thoracic and abdominal computed tomography scans. Patients' Karnofsky index had to be 60% or better. No systemic chemo-, and/or immunotherapy was allowed within 4 weeks prior to vaccination. Important exclusion criteria were evidence of CNS metastases, active autoimmune or infectious diseases, pregnancy and lactation, as well as significant psychiatric abnormality.

Peptide-pulsed dendritic cells were generated as previously described [15]. Briefly, PBMCs from leukapheresis were isolated on Lymphoprep™ (AXIS-SHIELD PoC AS, Oslo, Norway), frozen in aliquots and stored in liquid nitrogen. One week prior to vaccination, PBMCs were thawed, washed and cultured in medium containing gentamycin, glutamine and heat inactivated autologous plasma. On days 1 and 5, IL-4 and GM-CSF were added. To differentiate mature DCs, TNF-α and prostaglandin E_2 were added on day 6. On day 7, cells displaying phenotypical and morphological characteristics of mature DCs, i.e. a veiled appearance and ≥75% CD83 expression, were pulsed for 4 h with a modified survivin-derived HLA-A2 restricted $survivin_{96-104}$ epitope, LMLGEFLKL (30 μg/ml final dilution, Clinalfa, Läufelingen, Switzerland) [16]. Cells were only used for vaccination if microbial tests of samples taken from cultures on days 1 and 5 proved to be sterile.

Patients were vaccinated at 7-day intervals for the first two vaccinations followed by 28-day intervals for further vaccinations. A total of $10–20 \times 10^6$ mature, $survivin_{96-104}$-pulsed DCs were resuspended in PBS, containing 1% human serum albumin, and injected intradermally in aliquots of 1.5×10^6 DCs per injection site in the ventromedial regions of the thighs close to the regional lymph nodes. Limbs where draining lymph nodes had been removed and/or irradiated were excluded. Leukapheresis was repeated after five vaccinations in absence of severe deterioration of patient's state of health or occurrence of CNS metastases.

1.1.2. Measurement of clinical and immunological responses

CT scans were performed prior to vaccination and every 3 months thereafter or in case of severe clinical signs of disease progression.

Immunological responses were monitored by the ELISPOT assay, using PBMCs obtained before vaccination start and at the time of the fifth vaccination, to detect $survivin_{96-104}$-specific IFN-γ release [14]. To extend the sensitivity of the ELISPOT assay, PBMCs were stimulated once in vitro at a concentration of 1×10^6 cells per ml in 24-well plates (Nunc, Roskilde, Denmark) in X-vivo medium (Bio Whittaker, Walkersville, MD), supplemented with 5% heat-inactivated human serum and 2 mM of L-glutamine in the presence of 10 μM of peptide. Two days later, 40 IU/ml recombinant interleukin-2 (IL-2) (Chiron, Ratingen, Germany) were added. After 10 days, the cells were tested for reactivity. To this end, nitrocellulose bottomed 96-well plates (MultiScreen MAIP N45, Millipore, Glostrup, Denmark) were coated with an anti-IFN-γ antibody (1-D1K, Mabtech, Nacka, Sweden). Lymphocytes were added at $10^4–10^5$ cells in 200 μl X-vivo medium per well together with 10^4 T2-cells and the relevant peptides at a final concentration of 2 μM. After an overnight incubation at 37 °C and two washes, the biotinylated detection antibody (7-B6-1-Biotin, Mabtech, Nacka, Sweden) was added; its specific binding was visualised using alkaline phosphatase-avidin together with the respective substrate (GibcoBRL, Invitrogen Corporation, Carlsbad, CA). The reaction was terminated upon the appearance of dark purple spots, which were quantitated using the AlphaImager System (Alpha Innotech, San Leandro, CA).

As our previously performed experiments have demonstrated that the amount of background spots is highly comparable when either omitting a control peptide or using a known HLA-A2 restricted CTL epitope from HIV-1 $pol_{476-484}$ (ILKEPVHGV) in a large numbers of both healthy individuals and cancer patients of different origin, we renounced to

use irrelevant peptides for control in this setting. Therefore, the average number of survivin-specific cells was calculated after subtraction of spots without added peptide for each patient.

Survivin$_{96-104}$/HLA-A*0201 reactive CD8+ T lymphocytes were also tracked in situ both at the vaccination sites as well as in visceral, soft tissue, or cutaneous metastases by means of multimeric survivin$_{96-104}$/HLA-A*0201 complexes. Biopsies of vaccination sites were taken 24 h after intradermal injection in all patients, whereas metastatic lesions were only removed in selected patients, if easily accessible (patients KN and GB), or removed during a curative intent (patient WW). The staining procedure for multimeric peptide/MHC complexes has been described recently [16]. The specificity of these complexes has been confirmed using different antigens or MHC/survivin-peptide constructs in tumour samples obtained from HLA-mismatched patients [14]. The multimeric survivin$_{96-104}$/HLA-A*0201 complexes were generated by introduction of a recognition site for enzymatic biotinylation at the 5′ end of the extracellular domains of HLA-A*0201 (residues 1–275). The recombinant protein was purified by size-exclusion (Sephadex G25, Pharmacia, Erlangen, Germany) and ion-exchange (mono-Q, Pharmacia) chromatography and folded in vitro by dilution in presence of the respective peptides and β2-microglobulin. After gel filtration on a Sephadex G25 column, the protein was multimerised with streptavidin-FITC conjugated to dextran molecules (kindly provided by L. Winther, DAKO, Copenhagen, Denmark) to generate multivalent HLA-dextran complexes. Cryopreserved sections of the respective samples were dried overnight and subsequently fixed in cold acetone for 5 min. All incubation steps were performed in the dark at room temperature as follows: (i) 45 min of an anti-CD8 antibody (1:100, clone 1IT8a, Pharmingen, San Diego, CA); (ii) Cy3-conjugated goat anti-mouse (1:500 diluted; code 115–165–100, Dianova, Hamburg, Germany) for 45 min; and finally (iii) the multimers for 75 min. Between each step, the slides were washed twice for 10 min in PBS/BSA 0.1%. Finally, slides were mounted in vectashield and observed under a Leica Confocal Microscope (TCS 4D, Leica, Mannheim, Germany).

2. Results

2.1. Patient characteristics, clinical course and toxicity

Five far-advanced stage IV melanoma patients were enrolled, two suffering from uveal melanoma, one from soft tissue melanoma and the remaining two from cutaneous melanoma.

Due to manifestation of symptomatic brain metastases 4 weeks after initiation of therapy, one patient (PB) was taken off therapy, having received only two vaccinations. Another patient (WW), whose liver metastases could be stabilised under vaccination therapy, suffered from a myocardial infarction during a curative intent surgical resection of remaining liver metastases and a newly occurred adrenal metastasis, and died several days thereafter. The other three patients demonstrated slow progression of metastatic disease without substantial impairment in their general state of health. Remarkably, for patient KN, an overall survival of 13 months (from vaccination start to death) could be achieved despite a heavy metastatic load and fast disease progression at the start of vaccination. Patient GB remains still on protocol 14 months after initiation of vaccination with survivin-peptide-pulsed DCs. It should be noted, however, that two patients (RW and GB) received additional localised treatment for tumour control, either radiation of subcutaneous tumours (RW) or local chemotherapy (GB). Detailed patient characteristics, previous therapy, number of vaccinations and survival status are summarised (Table 1).

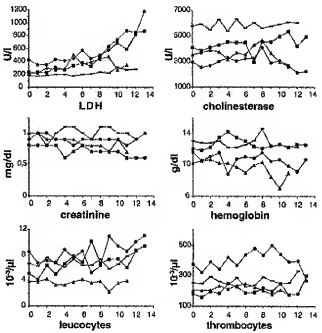

Fig. 1. Laboratory values. Stable detection of lactate dehydrogenase, cholinesterase, creatinine, hemoglobin, leucocytes and thrombocytes following vaccination therapy over months ((▲) RW; (●) KN; (▬) WWE; (■) GB).

No major toxicities occurred. Thus, hemoglobin, leucocytes and thrombocytes, as well as lactate dehydrogenase, creatinine and cholinesterase were not influenced by the vaccination therapy (Fig. 1). No signs of local toxicity were observed at the injection sites. Furthermore, there was no detection of impaired wound healing, hemorrhagic disorders, cardiac dysfunction, vasculitis or inflammatory bowel disease.

Survivin-specific CD8+ T cell responses. To monitor the course of the cytotoxic T cell responses, PBMCs obtained prior to and 3 months after vaccination were tested for reactivity to the modified survivin$_{96-104}$ epitope by ELISPOT for IFN-γ. Before analysis, PBMCs were stimulated once in vitro to extend the sensitivity of this assay. Although re-

Table 1
Patient characteristics

ID	Age/sex	Time from primary tumour to stage IV	Previous therapy	Measurable disease	Clinical outcome	No. of vaccinations	Survival after first vaccination[a]
GB	40/female	4 years, 8 months	LITT, fotemustine/IL-2/IFN-α, treosulfan/gemcitabine	Liver	PD (slow growth of pre-existing and new hepatic lesions, new pancreas and pleural metastases)	15	+14 months
KN	53/male	11 years	IL-2/IFN-α/histamine, treosulfan/gemcitabine	Liver, kidney, soft tissue, bone	PD (slow growth of pre-existing lesions, new lymph node, pleural and medias-tinal lesions)	13	13 months
WW	73/male	14 months	Surgery, DC-vaccination, dacarbazine	Liver	PD (stable hepatic, but new adrenal metastasis)	12	12 months due to post-surgical stroke
RW	72/male	16 years	Surgery, radiotherapy, adriblastin/ifosfamid, ivotect, dacarbazine, TNP/melphalan	Soft tissue	PD (growth of pre-existing and new soft tissue metastases; detection of heart, lung and muscle metastases after 12 vaccinations)	12	+12 months
PB	52/male	2 years, 3 months	Radiotherapy	Lung, kidney	PD (new skin and brain metastases)	2	4 months

[a] Cut of date: March 2003.

sponses measured directly ex vivo may reflect in vivo conditions more accurately as compared to ELISPOT analyses carried out after in vitro stimulation of T cells, this method is unsuitable for the majority of patients without prior IL-2 therapy, as no T cell responses can be detected by direct analyses in this group of patients in contrast to ELISPOT assays with prior short in vitro peptide T cell stimulation [17]. In all four patients tested, an induction of survivin$_{96-104}$ reactive T cells was evident (Fig. 2). Analysis for reactivity to other HLA-A*0201 restricted survivin peptides, i.e. the non-modified survivin$_{96-104}$ and the adjacent Sur$_{95-104}$ epitope, demonstrated a T cell response against these peptides in two of the patients (KN and RW) (data not shown).

The prognostic and clinical value of measurements of tumour-specific T cell responses in peripheral blood has been questioned repeatedly [18]; thus, we also tested for the presence of survivin$_{96-104}$/HLA-A*0201 reactive CD8+ T lymphocytes among tumour infiltrating lymphocytes in situ by peptide/MHC multimer staining. To validate the method, we first analysed tissue samples from delayed type hypersensitivity reactions occurring at the vaccination site within 24 h (Fig. 3a and b). This analysis confirmed earlier observations that intradermal injections of peptide-pulsed DC induce a strong peptide-specific inflammatory T cell infiltrate [16]. Subsequently, the peptide/MHC multimer staining procedure was applied on soft tissue (Fig. 3c and d) and visceral (Fig. 3e and f) metastases, which revealed the presence of survivin$_{96-104}$/HLA-A*0201 reactive cells among the CD8+ infiltrate. This observation suggests that the vaccination does not only induce T cells with the desired specificity, but also endows them with the necessary homing capacity.

3. Discussion

Strong and frequent expression are essential characteristics of antigens intended for therapeutic tumour vaccina-

Fig. 2. Analysis of immunity to survivin peptides assessed by IFN-γ ELISPOT. The numbers of IFN-γ spot-forming cells above background are depicted before vaccination (black bars) and at the time of the fifth vaccination (grey bars).

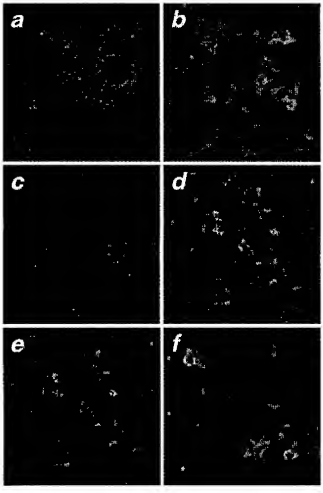

Fig. 3. In situ detection of survivin-reactive CTLs. Biopsies from the third vaccination obtained 24 h after DC injection (a and b), a soft tissue metastasis excised after 9 DC vaccinations from patient KN (c and d) and a visceral tumour manifestation removed after 12 vaccinations from WW (e and f) were subjected to Cy3-conjugated anti-CD8 antibodies (red) and FITC-conjugated survivin$_{96-104}$/HLA-A*0201 multimers (green). Magnification 200× a, c, and e; 500× b, d, and f.

tions [19]. A correlation of antigen expression with oncogenic phenotype would be an additional advantage since it avoids the selection of antigen-loss variants [20]. Survivin fulfils these criteria since it is over-expressed in most human cancers but nearly undetectable in normal adult tissues, as well as linked to apoptosis control and cell division [7]. Here, we report on a series of late stage IV melanoma patients who were vaccinated with an HLA-A2 restricted survivin epitope using DC as adjuvant. The epitope used was modified by replacing threonine at position 2 with methionine, a better anchor residue with improved binding affinity to the MHC molecule. Notably, our prior studies have demonstrated that CTLs, reactive to the modified survivin peptide, are capable of lysing melanoma cells in vitro [14]. Our data demonstrate that even in heavily pre-treated patients with far-advanced disease, survivin-specific T cell responses can readily be mounted within the pool of circulating lymphocytes. However, previous trials suggested that both the presence of peptide-specific CD8+ T cells in the peripheral blood and their homing to tumour lesions is essential for favourable clinical outcome [21]. In the case of our patients, induction of survivin-specific CTLs was demonstrated both by ELISPOT analysis in peripheral blood as well as by in situ survivin$_{96-104}$/HLA-A*0201 multimer staining of reactive T cells infiltrating metastatic lesions. Since cytotoxic T cells, recognising specific epitopes on tumour cells, are able to induce tumour lysis, the unexpectedly long survival of the four patients mounting a survivin-specific T cell response may actually be attributed to this response. In this regard, we recently demonstrated an association between tumour-specific T cell responses and clinical course [22]. It should be noted, however, that no decisive conclusions about clinical efficacy of survivin-based vaccination can be drawn from this series of patients treated with compassionate intend. The aim of our study was rather to evaluate the toxicity of survivin-based vaccinations. In fact, no therapy-induced side effects were observed. This may be explained by the negligible expression of survivin in normal tissues. However, since it was reported that survivin is expressed by vascular cells following vascular injury, special attention was directed to signs of vascular alteration, e.g. vasculitis or impaired wound healing [23]. However, neither clinical nor histological signs for such changes were detectable.

In summary, although this report does not represent a controlled clinical study, it indicates that vaccination with survivin-derived peptides may represent a promising and well-tolerated therapeutic option for patients with solid tumours which should be tested in controlled clinical trials. Indeed, such a trial for several different tumour entities in a second line setting has recently been initiated (Ref. No. 0899/01, Paul-Ehrlich-Institute, Langen, Germany).

Acknowledgements

We thank Claudia Siedel, Eva Fuchs, Claudia Kurzmann, Monika Ossadnik and Merete Jonassen for excellent technical assistance. We further extend our gratitude to all the patients who participated in this study and donated blood, tissue and tumour samples for analysis. This work was supported by the German Ministry for Education and Research (K.O., E.K.; grant BMBF 01GE0601 and IZKF-01KS9603) the Deutsche Krebshilfe (grant 10-1845-Be 1), the Danish Medical Research Council (M.H.S., P.t.S.), and the Wilhelm Sander-Stiftung (E.F.; grant Be-2000.033.1).

References

[1] Keilholz U, Gore ME. Biochemotherapy for advanced melanoma. Semin Oncol 2002;29(5):456–61.

[2] Niethammer AG, Xiang R, Becker JC, Wodrich H, Pertl U, Karsten G. A DNA vaccine against VEGF receptor 2 prevents effective angiogenesis and inhibits tumor growth. Nat Med 2002;8(12):1369–75.

[3] Jansen B, Wacheck V, Heere-Ress E, Schlagbauer-Wadl H, Hoeller C, Lucas T. Chemosensitisation of malignant melanoma by BCL2 antisense therapy. Lancet 2000;356(9243):1728–33.
[4] Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S. Mutations of the BRAF gene in human cancer. Nature 2002;417(6892):949–54.
[5] Nestle FO, Alijagic S, Gilliet M, Sun Y, Grabbe S, Dummer R. Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. Nat Med 1998;4(3):328–32.
[6] Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ. Immunobiology of dendritic cells. Annu Rev Immunol 2000;18:767–811.
[7] Altieri DC. The molecular basis and potential role of survivin in cancer diagnosis and therapy. Trends Mol Med 2001;7(12):542–7.
[8] Altieri DC. Validating survivin as a cancer therapeutic target. Nat Rev Cancer 2003;3(1):46–54.
[9] Grossman D, Kim PJ, Schechner JS, Altieri DC. Inhibition of melanoma tumor growth in vivo by survivin targeting. Proc Natl Acad Sci USA 2001;98(2):635–40.
[10] Islam A, Kageyama H, Takada N, Kawamoto T, Takayasu H, Isogai E. High expression of survivin, mapped to 17q25, is significantly associated with poor prognostic factors and promotes cell survival in human neuroblastoma. Oncogene 2000;19(5):617–23.
[11] Kawasaki H, Altieri DC, Lu CD, Toyoda M, Tenjo T, Tanigawa N. Inhibition of apoptosis by survivin predicts shorter survival rates in colorectal cancer. Cancer Res 1998;58(22):5071–4.
[12] Andersen MH, Pedersen LO, Becker JC, thor Straten P. Identification of a cytotoxic T lymphocyte response to the apoptosis inhibitor protein survivin in cancer patients. Cancer Res 2001;61(3):869–72.
[13] Schmitz M, Diestelkoetter P, Weigle B, Schmachtenberg F, Stevanovic S, Ockert D. Generation of survivin-specific CD8+ T effector cells by dendritic cells pulsed with protein or selected peptides. Cancer Res 2000;60(17):4845–9.
[14] Andersen MH, Pedersen LO, Capeller B, Brocker EB, Becker JC, thor Straten P. Spontaneous cytotoxic T-cell responses against survivin-derived MHC class I-restricted T-cell epitopes in situ as well as ex vivo in cancer patients. Cancer Res 2001;61(16):5964–8.
[15] Thurner B, Roder C, Dieckmann D, Heuer M, Kruse M, Glaser A. Generation of large numbers of fully mature and stable dendritic cells from leukapheresis products for clinical application. J Immunol Methods 1999;223(1):1–15.
[16] Schrama D, Pedersen LO, Keikavoussi P, Andersen MH, Straten PP, Brocker EB. Aggregation of antigen-specific T cells at the inoculation site of mature dendritic cells. J Invest Dermatol 2002;119(6):1443–8.
[17] Andersen MH, Gehl J, Reker S, Pedersen LO, Becker JC, Geertsen P, et al. Dynamic changes of specific T cell responses to melanoma correlate with IL-2 administration. Semin Cancer Biol 2003;13(6):449–59.
[18] thor Straten P, Guldberg P, Gronbaek K, Hansen MR, Kirkin AF, Seremet T. In situ T cell responses against melanoma comprise high numbers of locally expanded T cell clonotypes. J Immunol 1999;163(1):443–7.
[19] Parmiani G, Castelli C, Dalerba P, Mortarini R, Rivoltini L, Marincola FM. Cancer immunotherapy with peptide-based vaccines: what have we achieved? Where are we going? J Natl Cancer Inst 2002;94(11):805–18.
[20] Andersen MH, thor Straten P. Survivin: a universal tumor antigen. Histol Histopathol 2002;17(2):669–75.
[21] Berger TG, Haendle I, Schrama D, Lufl M, Bauer N, Pedersen L. Circulation and homing of melanoma-reactive T cells to both cutaneous and visceral metastases after vaccination with monocyte-derived dendritic cells. Int J Cancer 2004;111(2):229–37.
[22] Andersen MH, Keikavoussi P, Brocker EB, Schuler-Thurner B, Jonassen M, Sondergaard I. Induction of systemic CTL responses in melanoma patients by dendritic cell vaccination: cessation of CTL responses is associated with disease progression. Int J Cancer 2001;94(6):820–4.
[23] Blanc-Brude OP, Yu J, Simosa H, Conte MS, Sessa WC, Altieri DC. Inhibitor of apoptosis protein survivin regulates vascular injury. Nat Med 2002;8(9):987–94.